Electronic Supplementary Information

Dual-Fluorophore Ratiometric pH Nanosensor with Tuneable pK_a and Extended Dynamic Range

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Supplementary materials

Nanosensors

Acrylamide 99% minimum, ammonium persulsufate (APS), dioctyl sulfosuccinate sodium salt (AOT) and N,N,N,N-tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich (Gillingham, United Kingdom). N,N'methylenebis(acrylamide) and Brij 30® were obtained from Fluka Analytical (Gillingham , United Kingdom). Hexane HPLC grade and Ethanol analytical grade were obtained from Fisher Scientific (Loughborough, United Kingdom). Argon gas acquired from BOC Gases (Manchester, United Kingdom). Spectra/ Por® dialysis 6-8000 MWCO tubing purchased from Spectrum Laboratories, Inc. (California, United States of America). Deionised water (18.2 MΩ) generated by Maxima HPLC grade USF Elga.

Dyes

Fluorescein isothiocyanate dextran (FITC-D) was obtained from Sigma-Aldrich (Gillingham, United Kingdom), where as Oregon Green® succinimidyl ester, 5-(and-6)-carboxytetramethylrhodamine succinimidyl ester and amino dextran 10000 MW were obtained from Invitrogen™ (Paisley, United Kingdom).

Buffer solutions

Sodium phosphate dibasic (Na_2HPO_4), citric acid monohydrate and sodium borate decahydrate were obtained from Sigma-Aldrich (Gillingham, United Kingdom).

Equipment

Fluorescence, dynamic light scattering, and pH measurements were taken using Varian Cary Eclipse fluorescence spectrophotometer, Viscotek (802) and Jenway 3510 pH meter, respectively. SigmaPlot® (11.0) was utilized to analyze the data generated.

Supplementary methods

Dye conjugation

The method for dye to dextran conjugation was adapted from Zen et al. [1] Oregon Green® succinimidyl ester (0.005 g, 9.816 x 10^{-6} mol) and amino dextran 10000 MW (0.020 g, 2 x 10^{-6} mol) were dissolved in a sodium borate decahydrate buffer (5 ml, 0.05 M), adjusted to pH 9. The solution was initially stirred at room temperature for 3 hours, followed by 21 hours at 4 °C. The product, Oregon Green dextran (OG-D) was recovered through dialysis with deionised water, using 6-8000 MW size exclusion tubing, and freeze dried overnight to yield OG-D.

The conjugation method was replicated for 5-(and-6)-carboxytetramethylrhodamine succinimidyl ester (0.005 mg, 9.478 x 10^{-6} mol), to yield for 5-(and-6)-carboxytetramethylrhodamine dextran (TAMRA-D).

Preparation of nanosensors

Brij 30® (3.080 g, 8.508 mmol), AOT (1.590 g, 3.577 mmol) and deoxygenated hexane (42 ml) were stirred under argon for 10 minutes. Acrylamide (0.540 g, 7.579 mmol), N'methylenebis(acrylamide) (0.160 g, 1.307 mmol), OG-D (20 µl, 5mg/ ml) and TAMRA-D (90 µl, 5mg/ml) were dissolved in deionised water, made up to 2 ml. This solution was added to the stirring hexane surfactant solution and allowed to deoxygenate for a further 10 minutes. APS (30 µl, 10% w/v) and TEMED (15 µl, 0.1 mmol) were added to the stirring solution to initiate polymerisation. The mixture was left to stir for 2 hours under argon at room temperature. The resultant solution was a transparent solution. Hexane was removed via rotary evaporation. Ethanol (50 ml) was added to the suspension, producing a white opaque suspension. The suspension was washed using centrifugation (10 times, 6000 rpm, 10 minutes), using a Hermle (Z300) centrifuge. The pellet was light pink in colour, indicating the presence of TAMRA-D dye. The resultant solid was dried using vacuum filtration (200 nm pore filter, Sartorius Stedim Biotech) and left overnight in a desiccator. Yield based on starting amounts of acrylamide and bisacrylamide 60 %.

The pK_a of the nanosensors was adjusted by increasing the ratio of FITC-D with respect to OG-D, in the pH sensitive fluorophore proportion of the aqueous phase of the micro-emulsion during nanosensor preparation, as described in Table 1.

Percentage FITC-D with respect to OG-D (%)	Volume of pH sensitive fluorophores in 5 mg/ ml solutions		pK_a (mean, n=3))	Effective dynamic range (mean, n=3)
	FITC-D (µL)	OG-D (µL)		
0	0	20	4.81	1.09
25	5	15	5.04	1.54
50	10	10	5.54	2.01
75	15	5	5.9	1.76
100	20	0	6.38	1.27

Table 1: Volumes of FITC-D and OG-D used to tune pH sensitive nanosensors.

Buffer solutions

Universal buffer solutions of pH ranging between 2.5 to 8 were created using stock solutions of Na_2HPO_4 (200 ml, 0.2 M) and citric acid monohydrate (200 ml, 0.1 M). Volumes of each stock solution were taken as shown in Table 2 and diluted to 40 ml with deionised water.

Na_2HPO_4 0.2 M (ml)	Citric acid monohydrate 0.1 M (ml)	Buffer Solutions (pH) Data Set 1	Buffer Solutions (pH) Data Set 2	Buffer Solutions (pH) Data Set 3
2.16	17.84	2.53	2.52	2.62
4.08	15.92	3.04	3.05	3.10
6.04	13.96	3.51	3.51	3.56
7.72	12.28	4.02	4.08	4.09
9.00	10.90	4.52	4.55	4.57
10.28	9.72	5.02	5.05	5.09
11.36	8.64	5.53	5.53	5.56
12.84	7.16	6.01	6.08	6.13
14.20	5.80	6.52	6.49	6.52
17.44	2.60	7.01	7.01	7.05
17.98	2.03	7.52	7.48	7.50
19.53	0.48	7.99	8.02	8.09

Table 2: Volumes of Na_2HPO_4 and citric acid were diluted to 40 ml to produce buffer solutions between pH 2.5 and 8.

Modelling the response of pH-sensitive nanosensors

The pH calibration curve was modelled on Equation 1. [2, 3] Here I, I_{max} and I_{min} describe the experimental, maximum and minimum ratio response of a sensor, respectively. The slope factor 'b' (ln (1/ gradient)), represents the sensitivity of the sensor to changes in pH. Rearrangement of Equation 1, permits calculation of the pH (Equation 2) and pK_a (Equation 3) from experimentally determined fluorescence intensity measurements.

$$1) \quad I = I_{min} + \frac{I_{max} - I_{min}}{1 + e^{\frac{pK_a - pH}{b}}}$$

$$2) \quad pH = pK_a - ln\left(\frac{I_{max} - I_{min}}{I - I_{min}} - 1\right)^b$$

$$3) \quad pK_a = pH + ln\left(\frac{I_{max} - I_{min}}{I - I_{min}} - 1\right)^b$$

Inputting constants α, β, and γ simplifies Equation 1 to generate Equation 4. Equations 5 and 6 model the first and second derivatives of the calibration curves, respectively. The second derivative permits the calculation of the effective dynamic range. The effective dynamic range is calculated through subtraction of the pH values corresponding to the minima and maxima points using a graphical representation of the second derivative. Data presented in Figure 1 and 2a are produced from calibrations made in data set 1, Figure 2b is produced from calibrations of all three data sets and Figure 3 is produced using data set 2.

$$\alpha = I_{max} - I_{min}$$

$$\beta = e^{\gamma p K_a}$$

$$\gamma = \frac{1}{b}$$

$$4) \quad I = I_{min} + \frac{\alpha}{1 + \beta e^{-\gamma pH}}$$

$$5) \quad \frac{dI}{dpH} = \frac{\gamma\, \alpha\beta e^{-\gamma pH}}{(1 + \beta e^{-\gamma pH})^2}$$

$$6) \quad \frac{d^2I}{dpH^2} = \frac{\gamma^2\, \alpha\beta e^{-\gamma pH}(\beta e^{-\gamma pH} - 1)}{(1 + \beta e^{-\gamma pH})^3}$$

Dynamic light scattering

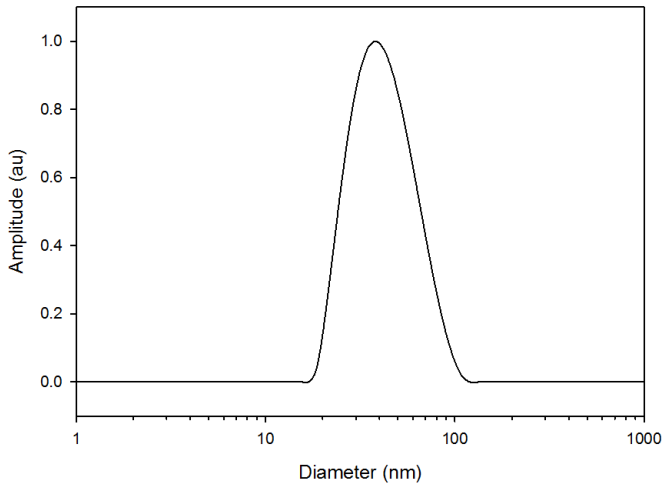

Figure A: DLS intensity distribution of FITC-D, OG-D (1:1 ratio) and TAMRA-D nanosensors, averaged over 100 measurements each 3 seconds in length at 25 °C and 10 % laser power). Particles have a mean hydrodynamic diameter of 43.8 nm.

Supplementary references

1. K. Zen, J. Biwersi, N. Periasamy and A. S. Verkman, *Journal of Cell Biology*, 1992, **119**, 99-110.
2. D. W. Hosmer and S. Lemeshow, *Applied Logistic Regression*, John Wiley & Sons, Massachusetts, 1989.
3. A. Delean, P. J. Munson and D. Rodbard, *American Journal of Physiology*, 1978, **235**, E97-E102.